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Anti-inflammatory and antioxidant effect of melatonin on recovery from muscular trauma induced in rats

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ABSTRACT

In recent decades, the number of people who practice sports has grown exponentially, increasing the number of muscular injuries. Trauma injury occurs when the muscle is exposed to a sudden compression force. Melatonin (MLT) has often been cited in the literature as a potent antioxidant and anti-inflammatory agent. This study was designed to evaluate MLT action on muscle tissue in Wistar rats in an experimental model of muscle trauma. Twenty-eight Wistar rats were used, divided into four groups: CO (Control), CO + MLT (Control + Melatonin), T (Trauma) and T + MLT (Trauma + Melatonin). MLT (20 mg/kg) was administered (ip) daily at dusk until day 7. The trauma occurred on day 1, 2 h before the first MLT application. On day 8, muscle tissue was collected for histological analysis (HE), immunohistochemistry (TNF-α and NFκB), evaluation of oxidative stress through analysis of lipoperoxidation by TBARS and activity of SOD and GPx enzymes, and analysis of nitrites and nitrates. In the evaluation of TBARS and SOD, we observed a significant increase in the T group and a significant decrease in the T + MLT group. In the evaluation of GPx, there was a significant increase in the T group and a significant decrease in the T + MLT group. The histological analysis of muscle tissue revealed structural changes of muscle fibers and inflammatory infiltrate in the T group but a decrease in this damage in the T + MLT group. In the immunohistochemical evaluation, increased expression of TNFα and NFκB proteins in the T group was observed and a significant decrease of this expression in the T + MLT group. MLT was shown to attenuate oxidative damage and to diminish the expression of inflammatory proteins and tissue damage in this experimental model.

1. Introduction

Skeletal muscle is one of the most abundant and superficial tissues of the human body and is thus exposed to various types of injuries. In sports, muscle injuries account for up to 55% of all injuries (Kääriäinen et al., 2000). The most common cause of injury in these cases is mechanical trauma, which often results in the athlete's inability to train or compete for several weeks (Almekinders, 1999; Järvinen et al., 2007).

To facilitate the study of muscle injuries, these types of injuries are commonly described as going through three distinct and overlapping phases. The first phase, considered “inflammation”, is characterized by rupture and necrosis of muscle fibers, hematoma and inflammation. The “repair” phase consists of phagocytosis of the necrotic tissue, activation of satellite cells and remodeling of damaged muscle fibers. Finally, the “fibrosis” phase, a period in which scar tissue reorganization occurs in areas where muscle regeneration was not effective (Järvinen et al., 2005; Tidball, 2017). The generation of free radicals and activation of the inflammatory process are the two main pathways that are engaged following the traumatic lesion.

Reactive oxygen species (ROS) are formed through toxic intermediates of ATP in mitochondria. Some of these ROS are also free radicals, substances considered extremely reactive by containing an

Abbreviations: C3-ohms, cyclic 3-hydroxymelatonin; CEUA, Committee on the Use of Animals; COX-2, cyclooxygenase-2; IL, interleukins; iNOS, inducible nitric oxide synthase; MCP-1, monocyte chemotactic protein; MLT, melatonin; NADPH, nicotinamide adenine dinucleotide phosphate; ROS, reactive oxygen species; TBARS, thiobarbituric acid reacting substances.

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unpaired electron in their last layer. An imbalance between oxidative and antioxidant substances generates oxidative stress. Oxidative stress is related to the production of tumor necrosis factor alpha (TNF-α) and nuclear transcription factor kappa B (NF-κB) in muscle injury, the latter being the main marker involved in the transcription of most cytokines and adhesion molecules involved in trauma recovery (Hu et al., 2007; Zeng et al., 2012). TNF-α and NF-κB are two important inflammatory markers that exert regulatory functions at all stages of muscle injury. TNF-α has some biological effects, such as the activation of macrophages and neutrophils and the increase of adhesion molecules involved in leukocyte activation, cell differentiation and apoptosis (Aggarwal, 2003). TNF-α is induced by a number of stimuli, including microorganisms, lipid mediators, tumor cells and cytokines. Its pro-inflammatory role is associated with the induction/production of interleukins (IL) and expression of adhesion molecules, as well as activation of apoptotic factors and regulation of the immune system during acute and chronic inflammation (Hu et al., 2007). Studies have shown that ROS can stimulate the activation of cellular transcription factors, including NF-κB. This controls the increased production of inflammatory cytokines, TNF-α and several interleukins (IL-1β, IL- 6, IL-8) that occur as a systemic inflammatory response. Thus, NF-κB serves as a marker sensitive to anti-inflammatory treatment (Cerqueira et al., 2005; Zeng et al., 2012).

Melatonin (MLT) is a lipophilic hormone synthesized and secreted primarily by the pineal gland. MLT is formed from serotonin through a reaction phase with the help of two enzymes, arylalkylamine *N*-acetyltransferase (AANAT) and hydroxyindole-*O*-methyltransferase (HIOMT). Serotonin acetylation occurs with the action of AANAT, which forms *N*-acetyl-serotonin, and HIOMT converts *N*-acetyl-serotonin into melatonin (Naseem and Parvez, 2014). Its secretion occurs exclusively at night, reaching peak plasma levels between 03:00 and 04:00. After the secretion of MLT, it is distributed through several body tissues, as it has high lipid solubility that facilitates its passage through cell membranes, including the blood-brain barrier (Acuña-Castroviejo et al., 2014, Marseglia et al., 2017).

MLT performs different functions, such as chronobiological, immunomodulatory, sedative, antitumor, analgesic, anti-inflammatory and antioxidant functions (Gitto et al., 2013; Naseem and Parvez, 2014; Moreira et al., 2015; Colares et al., 2016; Bona et al., 2018; Chang et al., 2018; Can et al., 2018). MLT has been studied in various diseases precisely because of its antioxidant and anti-inflammatory effects. When MLT acts as an antioxidant, it results in the formation of cyclic 3-hydroxymelatonin (C3-HOM), which then acts as an electron donor for radicals and eliminates two OH molecules, resulting in the formation of N1-acetyl-formyl-5-methoxykynurenamine (AFMK). MLT is a scavenger of numerous ROS, while activating various antioxidant enzymes, such as glutathione peroxidase, superoxide dismutase, and catalase (Carlomagno et al., 2018). Melatonin has been considered a potent antioxidant, and several of its metabolites are able to protect cells from oxidative damage and amplify antioxidant capacity (Naseem and Parvez, 2014; Can et al., 2018). As an anti-inflammatory agent, several studies have demonstrated that MLT acts by ceasing innumerable events within the cell cascade leading to the synthesis of pro-inflammatory substances, such as reducing the production of cytokines and chemokines and the recruitment of polymorphonuclear leukocytes to sites of inflammation (Poeggeler et al., 1993, Mutoh et al., 2007, Erdem et al., 2010; Gomez-Florit et al., 2013, Leon et al., 2014; Hardeland et al., 2018).

Muscle injuries have a large impact on the health system and need to be analyzed more deeply to find new therapies that can improve muscle regeneration.

Considering the need to elucidate the antioxidant and anti-inflammatory effects of MLT on muscle trauma, this study aims to evaluate the effects of MLT in an experimental model of muscle trauma in Wistar rats.

2. Materials and methods

2.1. Experimental design

The study was conducted at the Laboratory of Oxidative Stress and Antioxidants of the Lutheran University of Brazil (ULBRA) and had a qualitative and quantitative experimental design. To conduct the study, traumatic muscle injury was induced in Wistar rats.

2.2. Animals

Twenty-eight male Wistar rats, weighing 200–300 g, were used and kept in the vivarium of the Universidade Luterana do Brasil (ULBRA) from Canoas (RS). During the experiment, animals were kept in 47 cm × 34 cm × 18 cm boxes lined with wood shavings and on a light/dark cycle of 12 h at a temperature between 18 and 22 °C. Water and food were given *ad libitum*.

The experiment was approved by the Research Ethics Committee on the Use of Animals (CEUA) of the Lutheran University of Brazil (ULBRA), under protocol 2016/160, in compliance with ethical legal principles (Federal Law 11.7 94/2008) and European Community guidelines (EEC Directive of 1986; 86/609/EEC).

2.3. Sample size and experimental groups

To detect a difference of 1.5 standard deviation (large effect), evaluated by histological parameters, estimating $\alpha = 0.05$ and $\beta = 90\%$, we calculated that seven animals per group would be used.

Animals were randomly distributed into four groups: control (CO), control + melatonin (CO + MLT), trauma (T), and trauma + melatonin (T + MLT).

2.4. Experimental procedures

2.4.1. Experimental model of muscular trauma

For the induction of trauma, animals were weighed and anesthetized with a solution consisting of ketamine hydrochloride (95 mg/kg) and 2% xylazine hydrochloride (8 mg/kg) *via* intraperitoneal injection.

A simple contusion impact was induced in the right quadriceps muscle of animals in the T and T + MLT groups by means of a press developed by the Industrial Center of Teaching and Research Equipment Ltda. (CIDEP/RS, Brazil) in collaboration with the ULBRA Laboratory of Oxidative Stress and Antioxidants, as described by Filippin et al. (2009). The trauma was caused by a 0.459 kg metal piece falling through a metal rod from a height of 18 cm onto the middle of the quadriceps muscle. The kinetic energy derived from the impact was 0.811 J (Filippin et al., 2009).

2.4.2. Melatonin administration

The solution was prepared at the time of administration (ip). Melatonin (20 mg/kg per animal) was diluted in 5 μL of ethanol with 500 μL of NaCl (0.9%), and the illumination recommendations were respected during administration, which was performed two hours after the start of the experiment and daily on the subsequent six days (Grigorov et al., 2014). Melatonin was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.4.3. Euthanasia of animals and tissue collection

On day 8, animals were weighed and anesthetized following the same anesthesia protocol used for trauma induction. Animals underwent euthanasia by exsanguination (anesthetic overdose by ketamine and xylazine ip, using three times the usual dose), according to the euthanasia practice guidelines of the National Animal Experimentation Control Council (CONCEA, 2016). Fig. 1 shows a schematic of the experimental procedures. The right quadriceps muscle was rapidly dissected out and frozen in liquid nitrogen for further analysis. A fragment

GROUP	Day 1			Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
	Anesthesia	Trauma	Application							
CO	Yes	Simulated	Saline solution	Saline solution	Saline solution	Saline solution	Saline solution	Saline solution	Saline solution	Death
CO+MLT	Yes	Simulated	MLT	MLT	MLT	MLT	MLT	MLT	MLT	Death
T	Yes	Yes	Saline solution	Saline solution	Saline solution	Saline solution	Saline solution	Saline solution	Saline solution	Death
T+MLT	Yes	Yes	MLT	MLT	MLT	MLT	MLT	MLT	MLT	Death

Fig. 1. Experimental procedures in the different groups.

was immersed in 10% formaldehyde solution for histological analysis and immunohistochemistry.

After the tissue was collected, the carcasses were packed in white plastic bags, duly labeled and immediately stored in a freezer for later incineration, according to the standard procedure indicated by the institution.

2.5. Biochemical analysis

2.5.1. Homogenate

Muscle tissues were homogenized for 1 min in an ULTRA-TURRAX® disperser (IKA-Werke GmbH & Co. KG) at 4 °C using 1.15% KCl (5 mL/g of tissue) and phenylmethanesulfonylfluoride (PMSF) at a concentration of 100 mM. Next, the homogenates were centrifuged for 10 min at 3000 rpm in a refrigerated centrifuge (SORVALL Super T21, Kendro Laboratory Products, USA). The supernatant was removed and stored in microtubes. The samples were stored at −80 °C for later analyses (Llesuy et al., 1985).

2.5.2. Protein quantification

The Bradford method is the technique for determination of total proteins using the "Coomassie brilliant blue" dye BG-250. This method is based on the interaction between BG-250 dye and macromolecules of proteins containing basic or aromatic side chain amino acids. At reaction pH, the interaction between the high molecular weight protein and BG-250 dye causes the equilibrium of the dye to shift to the anionic form, which absorbs strongly at 595 nm and can be read spectrophotometrically (Bradford, 1976).

2.5.3. TBARS analysis

The amount of aldehydes generated by LPO is determined by measuring the amount of thiobarbituric acid reacting substances (TBARS). Thiobarbituric acid was added to the samples at 0.67%, whereas trichloroacetic acid was added at 10%. The samples were incubated at 100 °C for 15 min and centrifuged at 3000 rpm (1612.8 g) for 10 min at 4 °C. Absorbency was determined by spectrophotometry at 535 nm and the values expressed as nmol/mg prot (Buege and Aust, 1978).

2.5.4. Activity of antioxidant enzymes: SOD and GPx

The analysis of SOD activity was based on inhibition of the epinephrine reaction with superoxide radical, which could be detected spectrophotometrically at 480 nm with values expressed as USOD/mg prot (Misra and Fridovich, 1972). GPx activity was determined based on the consumption of nicotinamide adenine dinucleotide phosphate (NADPH) in the reduction of oxidized glutathione, which could be detected spectrophotometrically at 340 nm within two minutes with the values expressed as nmol/min/mg prot (Flohé and Günler, 1984).

2.5.5. Metabolites of nitric oxide (nitrites and nitrates)

Nitric oxide production was measured indirectly using the quantitative Griess colorimetric assay. This assay is based on enzymatic reduction of nitrates (NO_3) to nitrites (NO_2) in the presence of nitrate reductase and NADPH, with subsequent colorimetric determination of NO_2 by the Griess reagent (a mixture of sulfanilamide and (naphthyl) ethylenediamine specific for NO_2). Because NADPH inhibits the Griess reaction, it is necessary to oxidize all NADPH that was not used in the reduction of NO_3. This is achieved by adding nitrate reductase. The reading was performed in a microplate reader at 540 nm and the results are expressed as mmol/L (Granger et al., 1999).

2.6. Immunohistochemistry and quantification of TNF-α and NF-κB expression

TNF-α and NF-κB expression in muscle tissue was determined by immunohistochemical analysis. Antigen retrieval was performed using buffer at 60 °C, and endogenous peroxidase activity was blocked by incubation in absolute methanol. The slides were incubated with rabbit polyclonal antibody TNF-α (SC-52746) and NF-κB (p65) (SC-372) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 1:200 overnight at 4 °C. The slides were washed with buffer and incubated with the secondary antibody (anti -mouse IgG-HRP, anti-goat IgG-HRP, Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 1: 300 for 30 min at room temperature. The slides were examined by a pathologist, who was aware of the groups, using a microscope equipped with a digital analysis system including a Zeiss Axioskop 40 microscope (Oberkochen, Germany) connected by a Roper Scientific camera (Media Cybernetics, Rockville, USA) to a computer with an image capture software. The

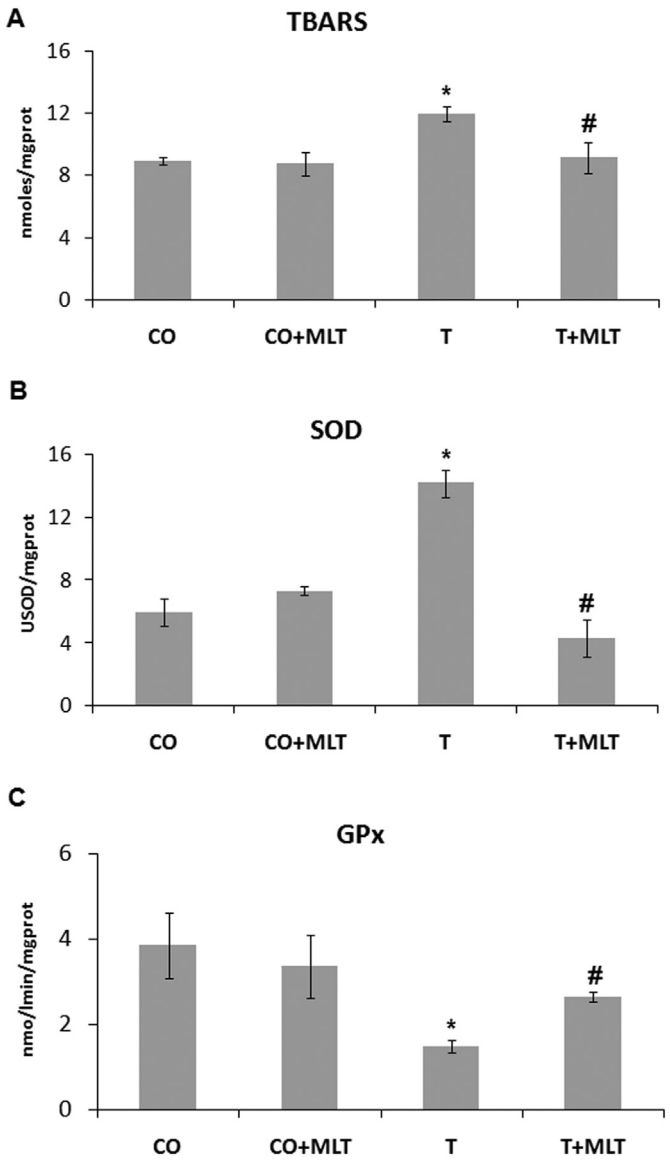

Fig. 2. TBARS levels (nmol/mg prot) and activity of the antioxidant enzymes SOD (USOD/mg prot) and GPx (nmol/min/mg prot) in the different experimental groups. Data are expressed as the mean ± standard error. Legend: CO: control, CO + MLT: control + melatonin, T: trauma, T + MLT: trauma + melatonin.

TBARS and GPx - * Significant difference between the T group in relation to the CO and CO + MLT groups ($p < .05$). # Significant difference between the T + MLT group and the T group ($p < .05$).

SOD - * Significant difference between the T group in relation to the CO and CO + MLT groups ($p < .001$). # Significant difference between the T + MLT group and the T group ($p < .001$).

Image-Pro Plus version 4.5 software (Media Cybernetics, Rockville, USA) was used to analyze digital images. Field photomicrographs of each slide (28 slides) were performed and the expression was determined by multiplying the mean image density by the percentage of positively stained areas (areas stained with brown spots). All images were 200× magnification.

2.7. Hematoxylin and eosin

For microscopic analysis, muscle fragments were fixed in 10% formaldehyde for 24 h and then embedded in paraffin. Next, the paraffin blocks were cut on a microtome (Leitz® 1512) into three microns (3 μ)

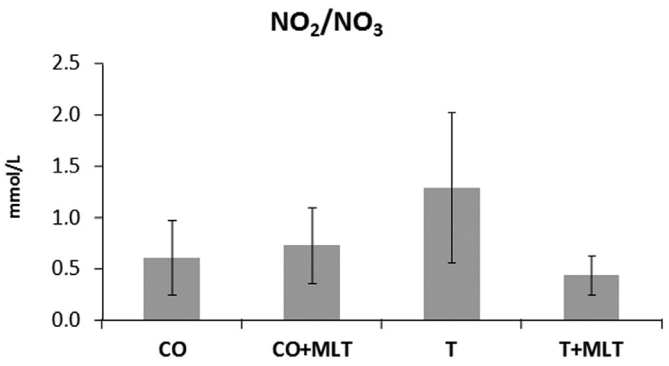

Fig. 3. Nitrite and nitrate levels (mmol/L) in the different experimental groups. Data are expressed as the mean ± standard error. Legend: CO: control, CO + MLT: control + melatonin, T: trauma, T + MLT: trauma + melatonin. There were no significant differences between the groups ($p > .05$).

slices and the slides were dipped in hematoxylin-eosin. The slides were evaluated by a blinded pathologist through a microscope equipped with a digital camera for image capture using Image-Plus software (Media Cybernetics, Bethesda, USA) at 200× and 400× magnification.

2.8. Statistical analysis

Data are expressed as mean ± standard error. Statistical significance was calculated using Graphpad Instat, version 3.0 for Windows. We used one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls test for multiple analysis. Results were considered statistically significant when $p < .05$.

3. Results

3.1. TBARS and antioxidant enzymes

Due to the involvement of oxidative stress in muscle trauma, we evaluated the oxidative damage in the quadriceps muscle using the thiobarbituric acid reactive substances (TBARS) technique (Fig. 2, A). We observed that in the trauma group (T), there was a significant increase in oxidative damage in relation to the control groups (CO and CO + MLT). The melatonin-treated group (T + MLT) showed a significant reduction in damage compared to that in the trauma group (T) ($p < .05$).

As the oxidative damage was increased in the quadriceps muscle of the animals in the trauma group (T), we analyzed the activity of the antioxidant enzymes superoxide dismutase (SOD) (Fig. 2, B) and glutathione peroxidase (GPx) (Fig. 2, C). In the SOD enzyme activity assay, we observed a significant increase in the trauma group (T) when compared to that in the control groups (CO and CO + MLT) and a significant reduction in the treated group (T + MLT) ($p < .001$). In the GPx enzyme activity assay, we observed a significant reduction in the trauma group (T) compared to that in the control groups (CO and CO + MLT) and a significant increase in the treated group (T + MLT) when compared to that in the trauma group (T) ($p < .05$). The results demonstrated that a reduction of oxidative stress occurred after treatment with melatonin due to its antioxidant effect.

3.2. Metabolites of nitric oxide (nitrites and nitrates)

The levels of nitric oxide (nitrite and nitrate) metabolites were evaluated in the quadriceps muscle (Fig. 3), but no significant differences were observed between groups ($p > .05$). The obtained results are possibly associated with the temporal parameters used in this model and experiment, as previously observed in other studies.

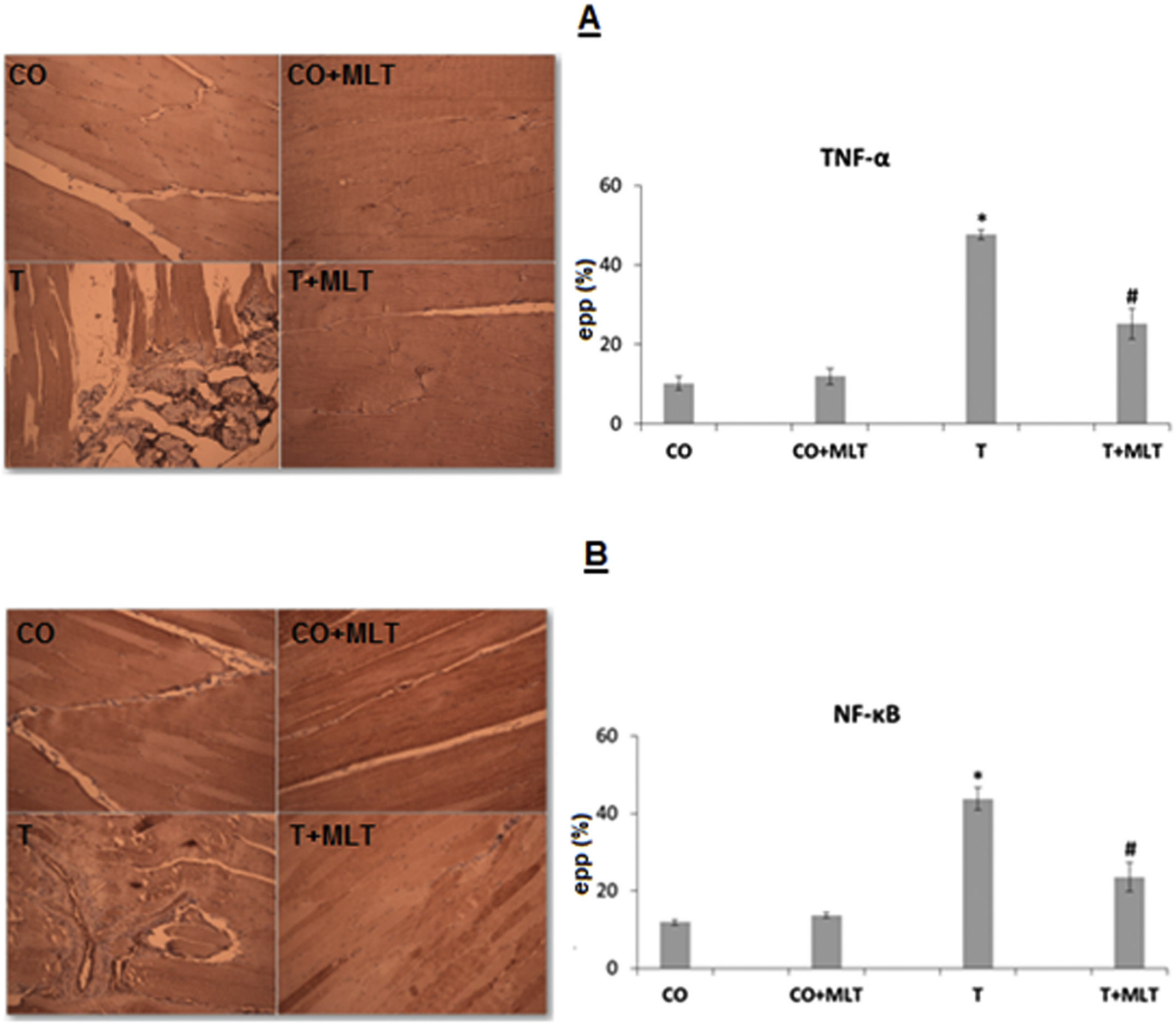

Fig. 4. Expression of tumor necrosis factor alpha (TNF-α) (A) and nuclear transcription factor kappa B (NF-κB) (B) in muscle tissue of animals subjected to muscle trauma and treated with melatonin. In the quantification, the data are expressed as the mean ± standard error. Magnification: 200×. Legend: CO: control, CO + MLT: control + melatonin, T: trauma, T + MLT: trauma + melatonin.
* Significant difference between the T group in relation to the CO and CO + MLT groups ($p < .001$). # Significant difference between the T + MLT group and the T group ($p < .001$).

3.3. Expression by immunohistochemistry of TNF-α and NF-κB

The inflammatory process involved in muscle trauma was assessed by tumor necrosis factor alpha (TNF-α) (Fig. 4, A), and nuclear transcription factor kappa B (NF-κB) (Fig. 4, B) levels that were determined by an immunohistochemical technique.

The expression of TNF-α and NF-κB was significantly increased in the trauma (T) group when compared to that in the control groups (CO and CO + MLT), and a significant reduction in expression in the treated group (T + MLT) occurred in relation to that in the trauma group (T). We suggest that melatonin acted by inhibiting/reducing NF-κB activation and consequently there was a reduction of cytokine TNF-α expression due to these anti-inflammatory effects of MLT.

3.4. Histological evaluation

Muscle tissue was assessed by HE staining and visual examination at 200× and 400× magnification levels (Fig. 5).

The structure of normal muscle fibers (CO and CO + MLT) is evidenced by their arrangement in parallel lines, with nuclei on their peripheries. In the T group, we observed tissue disorganization produced by trauma. Black arrows indicate inflammatory infiltrate. In the T + MLT group, tissue architecture was restructured, and the integrity of muscle fibers was preserved.

4. Discussion

It has been well established by different experimental approaches that inflammation results in oxidizing injury occurring in the presence of ROS; therefore, ROS play an important role in muscle injury. In

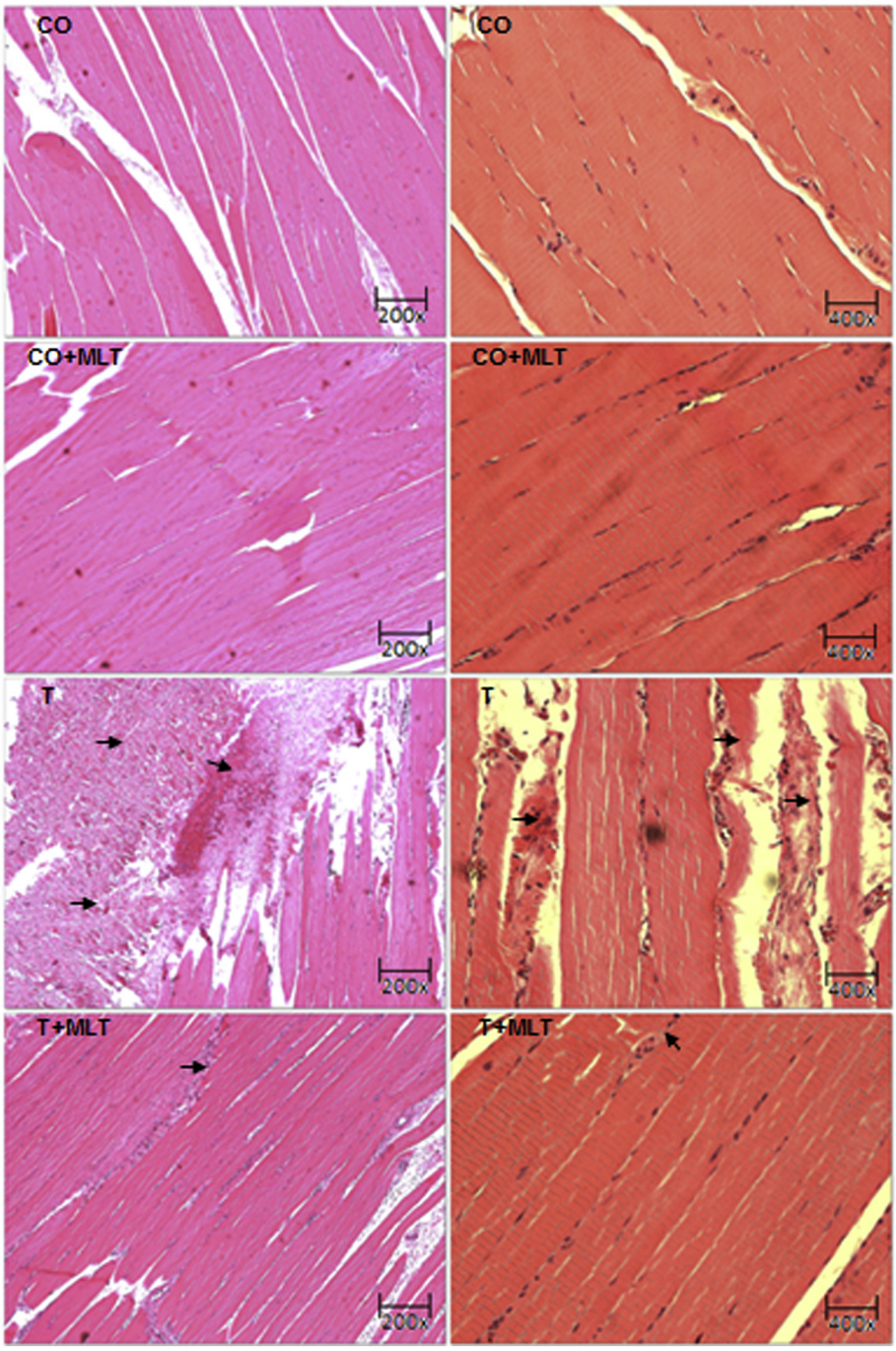

Fig. 5. Photomicrograph of muscle tissue at 200× (left column) and 400× (right column) magnification. In CO and CO + MLT, note the normality of the architecture of muscular fibers. In the images of group T, black arrows indicate inflammatory infiltrate. In T + MLT, muscle cells were restructured. Legend: CO: control, CO + MLT: control + melatonin, T: trauma, T + MLT: trauma + melatonin.

trauma models, the important role of ROS in modulating the inflammatory response has also been observed (Cuzzocrea et al., 2004).

In the present study, it was shown that trauma caused a series of alterations in the injured muscle, as evidenced by increased lipoperoxidation, increased SOD activity and decreased GPx activity. There was increased expression of the inflammatory proteins TNF-α and NF-κB, in addition to disorganization and disarrangement of muscle fibers with accumulation of inflammatory infiltrate, as demonstrated in the histological analysis.

The evidenced oxidative stress is a potent pathogenic factor that is connected to several degenerative diseases, including trauma to skeletal muscle.

We have shown a redox imbalance evidenced by increased superoxide anion radicals and as a response to increased SOD activity.

Increased lipoperoxidation can trigger activation and proliferation of satellite cells as a result of oxidative stress. Macrophages required for the lesion site become more likely to be stimulated to produce cytokines, such as TNF-α, increasing the inflammatory process and inducing apoptosis. In addition, lipoperoxidation reduces the activity of the mitochondrial respiratory chain, producing more ROS and increasing oxidative stress (Li et al., 2015).

In this study, MLT was used as a treatment option since different studies with different experimental models have used melatonin as an antioxidant agent.

Melatonin's protective effect against damage to muscle and membrane structure is more effective than that of other antioxidants. An article by McGinley et al. (2009) showed that the protective effect of antioxidants on muscle damage indicated that they can protect against strength loss resulting from fatigue, rather than protection against damage to muscle structure or membrane. The authors suggested that long-term antioxidant supplementation, particularly with vitamin E, in high doses can increase mortality and membrane damage. They state that antioxidant supplementation may not only fail to protect against damage but also actually interfere with cell signaling functions. Therefore, by ingesting antioxidant vitamins in their diet in an attempt to improve muscle performance, these individuals may actually slow down the adaptive processes to exercise. In this study, MLT was associated with a decrease in muscle damage, as lipoperoxidation levels were higher in the T group and reduced in the MLT-treated group, which suggests a protective effect on cell membranes.

SOD is an enzyme that has isoforms located intracellularly in the mitochondria and cytoplasm, playing an important role against oxidative stress (Shimizu et al., 2010). The findings of the present study indicated a reduction of SOD activity in the MLT-treated group. Ribeiro et al. (2016) obtained a reduction in SOD levels with seven days of infrared treatment in a model of muscular atrophy in rats. Park et al. (2013) and Martins et al. (2016) observed improvements in SOD levels with the use of melatonin in an *in vitro* experiment using muscle cells from rats atrophied by immobilization.

The redox balance in tissues depends on the proper functioning of the glutathione antioxidant system. The GPx enzyme oxidizes the reduced form of glutathione (GSH) to neutralize lipoperoxidation and the action of free radicals. Increased GPx activity was recorded in the T group. MLT administration in rats with induced trauma resulted in a significant reduction in enzyme activity.

Nitrite/nitrate levels did not differ significantly between groups in the experimental time period. A study by Kerkweg et al. (2006) evaluated local and systemic formation of ROS and nitric oxide at 5, 45 and 180 min after induction of blunt trauma to the quadriceps muscle of rats and demonstrated that local ROS formation in injured muscle began immediately after induction of the mechanical trauma, as indicated by changes in the glutathione redox state. Nitrite/nitrate levels, however, were not increased at these times. Filippin et al. (2009) also obtained similar results to those of this study in work with mechanical trauma induced by a press in rat muscle after 7 and 14 days of low-level Ga—As laser treatment (wavelength of 904 nm, average power of 45 mW, for 35 s). They reported ROS formation and increased iNOS, although nitrite and nitrate levels were not increased.

TNF-α and NF-κB expression observed by immunohistochemistry was significantly reduced in the group receiving MLT. These findings corroborate authors who demonstrated that MLT can act as an inhibitor of TLR4, which acts as an inhibitor of NF-κB transcription and as an inhibitor of TNF-α action through various mechanisms (Mutoh et al., 2007; Liu et al., 2012; Leon et al., 2014; Haddadi and Fardid, 2015; Maarman and Reiter et al., 2018).

Specifically, concerning TNF-α, Park et al. (2013) showed that melatonin was able to reduce its levels and also raise SOD levels in rats with muscle atrophy by immobilization. Borges et al. (2015) obtained similar results concerning TNF-α and decreased lipoperoxidation in rats treated with melatonin for muscle damage after ten days of strenuous exercise.

The increased NF-κB expression in the T group is related to the inflammatory process, leading to muscle protein damage. The use of MLT reduced its expression. Shen et al. (2013) observed a reduction in NF-κB expression by the application of a phenolic compound isolated from propolis in an eccentric muscle injury protocol in rats. In the same study, the NF-κB reduction consequently also generated a reduction of several cytokines and inflammatory markers, such as cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), IL-1β and monocyte chemotactic protein (MCP-1).

The histological evaluation of the quadriceps muscle of the T group at 200× and 400× magnification showed disarrangement and tissue disorganization with inflammatory infiltrate. Such evidence may be associated with increased lipoperoxidation, which promotes instability in muscle cell membranes with consequent rupture and extravasation of the intracellular fluid. In the MLT-treated group (T + MLT), there was less tissue damage and decreased inflammatory infiltrate with fiber rearrangement. Such evidence, together with the low lipoperoxidation levels observed, suggested the muscle-protective effect of MLT as it modulated parameters of oxidative and inflammatory stress and preserved the integrity of muscle cell membranes. Mehanna et al. (2017) evaluated the effects of MLT on muscle regeneration following an open lesion of soleus muscle in rats and found that treatment with MLT restructured muscle fibers by reducing inflammatory infiltrate with similar results to our study.

In sum, the findings of the present study indicated that melatonin was effective in muscle restoration in an experimental model of induced muscle trauma in rats, and it may be suggested as a treatment option for attenuation of muscle trauma.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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